P1093 Determination of Postantibiotic Effects *In Vitro* of Antituberculous Drugs Against *Mycobacterium tuberculosis* Using Radiometric Culture (TB-Bactec) System

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Objectives: The postantibiotic effects (PAE) *in vitro* of antituberculous drugs, including rifampicin, isoniazid, streptomycin, ethambutol, amikacin and various quinolones, alone and in combinations against isolates of *Mycobacterium tuberculosis,* were determined using the radiometric culture (TB-Bactec) system.

Methods: Known concentrations of isolates of *M. tuberculosis* were exposed to the antituberculous drugs of serum therapeutic concentrations for two hours, the antimicrobials were then removed by dilution and the treated and untreated (control) organisms were subsequently inoculated into the TB-Bactec 12 B medium and onto Middlebrook 7H11 agar slants. PAE were determined as the delay of growth by comparing the treated organisms to the controls where the growth were measured by the growth index in the radiometric culture system and by viable counts on the agar slants.

Results & Conclusions: Various antituberculous drugs, alone or in different combinations, have demonstrated significant PAE *in vitro* irrespective of the methods used for growth measurement. For *M tuberculosis,* although the viable count method reported PAE of drugs in terms of hours, the method is labour intensive and the reading can be erratic due to clumping of organisms, while PAE obtained by the radiometric culture method, though in terms of days, were less erratic and less labour intensive, yet contributing significantly to the design of treatment regimens since antituberculosis treatments are generally long-term and given daily or in alternate days.

P1094 Susceptibility of *M. avium* Complex (MAC) Isolates Tested During the Period 1993–1996

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Objective: To assess the in vitro susceptibility of MAC Isolates recovered from 1993–1996.

Methods: Over a four year period, we tested and monitored the susceptibility of 152 MAC isolates recovered from blood (96), respiratory (44) and tissue (12) specimens. Susceptibility was determined by an in-house breakpoint methodology using the BACTEC (Becton Dickinson, Sparks, MD) system. The drugs and (breakpoint concentrations – μg/mL,) used were amikacin (8.0); ciprofloxacin (8.0); clarithromycin (4.0), clofozimine (2.0); EMB (8.0); and rifabutin (1.0). The latter compound was initially tested at 0.5 μg/mL and in 1994 changed to 1.0 μg/mL.

Results: During the four year study period, the percentage of isolates susceptible to all drugs remained relatively constant and was 31.3%, 36.4%, 28.9%, and 34.2%, respectively. However, we observed an increasing number of isolates resistant to ciprofloxacin; 3 in 1993 and 1994; 7 in 1995; and 11 in 1996. There were 24 patterns of multiresistance (2–4 drugs) with no one pattern predominating; none of the 152 isolates were resistant to all drugs tested. Amikacin was present in 9 of the 24 patterns, whereas ciprofloxacin was present in one-half.

Conclusion: We believe the increasing use of ciprofloxacin in the treatment of MAC infections may be responsible for the observed increase in resistance to this drug.

Urinary tract infection: Laboratory diagnosis

P1095 Comparison of Chromagar Orientation a Chromogenic Medium, with Traditional Media for Culture of Urine Specimens

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CHROMagar Orientation, (CHROMagar Company, France), a chromogenic medium for direct identification of *E coli,* enterococci and *Proteus sp,* was compared to traditional culture media (MacConkey agar, CLED agar from Becton Dickinson and trypticase soy agar with horse blood) and CPS ID2 (bioMerieux, France) for isolation of bacteria from 642 urine specimens. Isolates from the traditional media were identified with API Strips (bioMerieux France).

Isolates from chromogenic media were identified by colony color, indole test and TDA test or API Strips. 165 cultures (25.70%) contained more than 10^4 CFU/ml of bacteria or yeast and 18 cultures contained 2 different pathogens. Correlation in enumeration was very good for the different methods (Chromogenic or classical media). The discrepancies were irregular and minor, always 1 log. or less.

Sensibility and specifity of the identification were 100% for *Proteae* taxa (TDA+) and enterococcal taxa (gram positive cocci and B-glucosidase+) and 97.90% for *E coli* (B-glucuronidase+, indole+). Most common urinary tracts pathogens, *E coli,* Enterocci and *P. mirabilis* are easily identified on CHROMagar Orientation. This new medium for bacterial examination of urine appears satisfactory for routine use and is more convenient than classical method by allowing pre-identification at the isolation step.

P1096 CHROMagar Orientation, CPS ID2, and Conventional Media for the Recovery of Micro-Organisms from Urines

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Objectives: To compare conventional plating media with two chromogenic media for clinical urine specimens.

Methods: 658 clinical urine specimens (10 μl each) were plated in parallel on BBL CHROMagar Orientation (= CO; Becton Dickinson [= BD]), CPS ID2 (= CP; BioMérieux), Columbia Agar W/5% sheep blood (BD), and MacConkey Agar W/O crystal violet (UNIPATH). Plates were incubated overnight (36°C). The two chromogenic media were read according to the manufacturers' instructions (including supplemental tests). Biochemical tests were used to clarify discrepant results and for the identification of all isolates from Columbia and MacConkey agars.

Results: 1 strain of *E. coli* (n = 275) on CO and 23 strains on CP did not produce the expected rose color. More enterococci (n = 280) were detected on CO and CP than on conventional media. On CO, 63 out of 66, and on CP 58 out of 64 isolates with large blue colonies were confirmed as *Klebsiella-Enterobacter-Serratia.* All *Proteus* isolates were correctly identified by the colony color on CO and CP. CO also allowed the detection of 9 of 12 isolates of *St. saprophyticus* (small rose-white colonies) whereas all staphylococci were white on CP.

Conclusions: Due to the slightly better identification rate of E. coli and the stronger blue coloration of respective colonies, CO performed slightly better than CP: Generally, chromogenic media are advantageous to conventional media for urine diagnosis.

P1097 Comparison between CPS ID2 Medium and CHROMagar Medium for Detection and Identification of Urinary Tract Bacterial Isolates

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Objectives: CPS medium (bioMerieux, France) and CHROMagar (CHR) medium (CHROMagar Company, France) were evaluated for detection and identification of the main bacteria responsible for urinary infections.

Methods: A 0.0075 ml volume of each urine samples was inoculated onto two media by urine robot (p.b.i. International, Italy), an automatical system used for streak plate method. Incubation was carried out for 20 h at 37°C. Isolates were identified on the basis of distinct colour exhibited by same species, together with a few extra tests. Any questionable results were confirmed with conventional methods.

Results: A total of 234 isolates, from 206 positive samples were screened. Apart from 5 isolates on CPS medium and 3 isolates on CHR medium, the counts of all of bacteria ($>10^4$ CFU/ml) had a good agreement onto two medium. Of the *Escherichia coli* strains tested (n = 107), 88.8% produced a pink to bordeaux colony on CPS medium, and 11.2% failed to produce β-D-glucoronidase, while 98.2% produced a pink colony on CHR medium and only 1.8% were ONPG negative. *Proteus mirabilis* (n = 2) and *Morganella morganii* isolates (n = 2) produced a clear to hazel colony with brown pigment halo, onto two medium. Similarity in colour didn't permit to discriminate between *Klebsiella, Enterobacter,* and *Citrobacter* species onto two media, but differentiated these from other members of *Enterobacteriaceae. Pseudomonas aeruginosa* isolates (n = 11) were easily differentiated from members of the *Enterobacteriaceae* but weren't distinguishable from nonmembers of the *Enterobacteriaceae.* All *Enterococcus* isolates (n = 36) produced a blue colour and grew as pinpoint isolated colonies onto two media, similar to *Streptococcus agalactiae* isolates (n = 4). *Staphylococcus aureus* (n = 5) produced a white to yellow colour on CPS medium and a white colour on CHR medium similar to *Staphylococcus epidermidis* isolates.

Conclusions: Two media permitted a good detection of single or mixed bacteria in culture. The distinct colour exhibited by some species allowed a fast presumptive identification of the main bacteria responsible of urinary infections. Two media appeared to be well suited for a screening laboratory procedure.

P1098 Uriselect 3®: A New Identification Medium for Laboratory Diagnosis of Urinary Tract Infections

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This study, conducted on 202 samples of infected urine (selected by cytology and results of biochemistry dipsticks), evaluated the performance of rapid identification of urinary bacteria using the nonselective Uriselect 3® agar medium (Sanofi Diagnostics Pasteur) compared to the technique usually used in the Tourcoing Hospital Laboratory (BCP medium and IMVIC tests). By detection of β-glucuronidase, β-glucosidase, indole and tryptophan deaminase, Uriselect 3® medium allows direct identification of *Escherichia coli, Proteus mirabilis,* and group D Streptococci, and orientation for the diagnosis of indole-positive *Proteus* and *Klebsiella-Enterobacter-Serratia* (KES group).

Materials and Methods: Plates and tests were carried out according to the manufacturer's instructions. On the first 100 urine samples, the bacteria counting technique was also evaluated in comparison with the use of a dipslide (Uricult® -ORION).

Results: Among 202 urine samples, 173 were monomicrobial (85.6%) and 129 of these isolates belong to the *Enterobacteriaceae* family. On Uriselect 3®, direct identification was obtained for 102 (79.1%) of these microorganisms of which 82 (63.6%) were *E. coli.* Six negative β-glucuronidase *E. coli* strains (6.8% of the total strains of *E. coli*) were unable to be identified on the Uriselect 3® medium. Nineteen urine samples (11%) revealed the presence of group D Streptococci. 26 urine samples presented a polymicrobial flora. Both techniques gave the same results for 20 mixtures. Among the isolates, 60% were identified directly on Uriselect 3®. In 3 cases, the growth of yeasts was only obtained on Uriselect 3®.

Conclusion: The performance obtained with Uriselect 3® is very satisfactory in terms of reliability. (counting and identification), speed of reading and legibility.

P1099 Diagnosing Paediatric Urinary Tract Infection: Dipstick versus Culture

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Objectives: To determine the clinical significance of dipstick-negative bacteruria in symptomatic children.

Methods: During 1994 all urine specimens from symptomatic children aged 0–16 years were examined by dipstick analysis and culture. Urine reagent strips for leucocyte esterase, blood and nitrite were used (Multistix 8SG, Ames) and read by the Clinitek System photometer (Ames). Dipstick analysis was considered negative if all 3 tests were non-reactive. Urines were cultured using a semi-quantitative technique. Culture-positive was defined as a pure or mixed growth of $>10^5$ colony forming units (CFUs)/ml or 10^4–10^5 CFUs/ml of a Gram-negative isolate. Bacteruria was considered significant if culture positivity was confirmed on repeat testing. Appropriate renal tract investigations were then performed.

Results: 1009 samples were examined. 168 (15.2%) were culture positive of which 28 (2.5%) were dipstick-negative. Four of these cases had bacteruria confirmed and one was found to have bilateral vesicoureteric reflux. Sensitivity, specificity, positive and negative predictive values of dipstick analysis versus culture were 83%, 61%, 28% and 95% respectively.

Conclusions: Reliance on dipstick testing alone will miss 1 in 20 children who warrant follow-up.

P1100 The Leukocyte Esterase Test Revisited: What Is Its Real Utility for Urine Screening?

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Objectives: To compare the leukocyte esterase test (LET) with microscopic examination (ME) of urine samples sent for culture to the microbiology laboratory.

Methods: 500 consecutive urines were analyzed for LET using biochemical test strips, and for ME of leukocytes. For ME, 50 μl of each urine sample was mixed in a corresponding well of a microtiter plate with 100 μl of formalin/safranin and examined at 400 × magnification on an inverted light microscope. Pyuria was considered when >40 leukocytes/mm^3 was detected.

Results: A total of 492 urine samples were evaluable for comparing results obtained by both methods. Pyuria was detected in 147 (40%) samples by ME but only in 110 (22.3%) by LET. In 18 (3.6%) urine samples the LET was positive but no pyuria was detected by ME. The percentage of sensitivity and specificity of LET was 47% and 94% respectively. In 27 urine samples, $>10^5$ CFU/ml of a single

organism grew on agar plates being the LET negative but having pyuria as detected by ME.

Conclusions: ME is the reference method for detecting pyuria, it is easy to perform also with a great number of urine samples and it is a cost-saving technique. LET is an expensive technique, easy to perform, highly specific, but lack of enough sensitivity by which it should not be used as screening method for selecting urine samples for culture.

P1101 Validation of 1 μl and 10 μl Calibrated Loops and Dipslide as Compared to 100 μl Pipette for Detection of Low Count Bacteriuria

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Objectives: To evaluate the ability and precision of various routine culture methods to detect the number of bacteria present in urine with respect to diagnosis of urinary tract infection (UTI).

Methods: *E. coli* ATCC 25922, *Ps. aeruginosa* ATCC 27853, *E. faecalis* ATCC 2912 and a laboratory strain of *S. saprophyticus* were used throughout the study. The following dilutions of each of the 4 bacteria were prepared: 10^2, 10^3, 10^4, 10^5 and 10^6 cfu/ml in urine, and for each dilution 20 subcultures onto 5% blood agar plates performed with each of the following: 1 μl and 10 μl calibrated plastic loops (NUNC, Denmark), dipslide (Uricult, Orion, Finland) and 100 μl pipette (Finnpipette). After incubation for 24 h plates were counted. The contents after dipping in water for the 10 μl loop and the 100 μl pipette as well as the dipslide after scraping off the agarsides were weighed on a precision scale.

Results: Variation coefficients correlated with volume of subculture but not for type of bacteria: For 10^3 cfu/ml: 1 μl loop: 46.2–76.6%; 10 μl loop: 13.3–21.9%; 100 μl pipette: 5.9–11.1%; dipslide: 13.3–36.2%. The 10 μl loop overestimated the counts with 50%, corresponding to the weight of water taken up by the loop weighing a mean of 15.24 μg (SD 1.55) in stead of 10 μg. Each agarslide on the dipslide absorbed approx. 200 μg of water, but bacterial counts equivalated that of the 10 μl loop.

Conclusion: The 1 μl loop should only be used for counts $>10^4$ cfu/ml, while the 10 μl loop can be used for counts $>10^3$ cfu/ml; neither can be used for precise counting of bacterial contents in fluids; this should only be performed with a 100 μl pipette.

P1102 Evaluation of an Automated Bacteriuria Screening System in Samples Collected with Bacteriostatic Substances

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Objectives: To evaluate an automated system for bacteriuria screening using the laser light scattering method (Uro-Quick Alifax) processing urine collected with bacteriostatic substances.

Methods: We chose a closed collection system (BD Vacutainer containing boric acid as bacteriostatic) to guarantee the quality of the samples and the standardization of results. The instrument is equipped with a dedicated software realized to interpret the lag phase induced by the presence of boric acid. 642 samples were tested with a traditional cultural method and with an automated system.

Results: We considered two different thresholds of positivity:

(a) 10^3 CFU/ml in which we obtained a sensitivity of 93.36% and a specificity of 93.75%;

(b) 10^5 CFU/ml with 99.29% of sensitivity and 98.28% of specificity.

Conclusions: These preliminary results show that urine samples collected with bacteriostatic substances should be processed with an acceptable grade of sensitivity and specificity.

P1103 Automation in Urinalysis by Flow Cytometry

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Objectives: 1) comparing results of UF-100 (Sysmex), a fully automated urine cell analyzer, with the results of urine sediment microscopy. 2) comparing culture results with bacterial count on UF-100.

Methods: 524 urines were aliquoted in 3 portions (1 for UF-100, 1 for microscopy and 1 reserve). Results of microscopy and UF-100 were compared for RBC, WBC, casts, yeasts and crystals. Discrepancies were reviewed by examination of the reserve aliquot. 396 urines were cultured on a Mc Conkey and a Blood Agar and reported as sterile, no infection or significant growth.

Results: 1) comparison microscopy – UF-100: 78.6% complete concordance, 21.4% discordant results (18.7% with review alarm, 2.7% without review alarm). 19.6% of the urine samples still have to be reviewed by microscopy. 2) comparison culture – bacterial count UF-100: The criteria used on UF-100 data to distinguish a urine to be cultured or not, are: bacterial count >250/μl or H-bacterial count >2 500/μl or WBC >16.5/μL. With these criteria it is possible to cancel 48.7% of the cultures with only 1% false negative results.

Conclusions: The UF-100 urinanalyzer is a good alternative for microscopic sediment analysis with more reproducable and objective results. The automate also seems to open the possibility for reducing the number of urine cultures.

P1104 Benefiting from the Urine CRP Levels in Localization of Urinary Tract Infections

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Objectives: We aimed to establish the important of CRP levels of blood and urine in localization of urinary tract infections.

Methods: This study has been constructed totally 104 patients defining as pyelonephritis (n = 59) and cystitis (n = 45). Forty normal cases have been studied as control group. CRP levels of blood and urine have been examined with semiquantitative latex agglutination method.

Results: The blood CRP levels on the 28 of 45 patients defining as cystitis were below 12 mg/L with 70% sensitivity, 62.2% specificity, 62.2% positive predictive value and 56% accuracy. The urine CRP levels on 36 of these cases were below 12 mg/L with 90% sensivity, 80% specifity, 80% positive predictive value and 75.8% accuracy.

The blood CRP levels only 4 of 59 patients defining as pyelonephritis were below 12 mg/L with 82.3% sensitivity, 90.3% specificity, 94.9% positive predictive value and 84.8% accuracy. The urine CRP levels on 4 of these cases below 12 mg/L with 92% sensitivity, 73.4 specificity, 77.9% positive predictive value and 82.8% accuracy.

Conclusions: The urine CRP levels was important as blood CRP levels in localization of urinary tract infections. It is noninvasive, simple and cheap.

P1105 Urinary Tract Isolates of *Haemophilus parainfluenzae* are Distinct by Repetitive Extragenic Palindromic (REP)-PCR and Cellular Fatty Acid (CFA) Analysis

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Objectives: To assess heterogeneity of *Haemophilus* spp. using REP-PCR and cellular fatty acid analysis.

Methods: We defined *Haemophilus* spp. isolated from an adult predominantly male population by biochemical profile, site of isolation, REP-PCR pattern and CFA analysis (MIDI system).

Results: In contrast to *Haemophilus ducreyi*, which was homogeneous by all measures, both *H. parainfluenzae* and *H. influenzae* were heterogeneous. However a group of urinary tract isolates of *H parainfluenzae* clustered by CFA analysis at a Euclidean distance of 6 and showed a single PCR pattern and only a few biochemical profiles. These isolates occurred as both pathogens and normal urethral flora. *H parainfluenzae* from other sources gave about ten PCR patterns and CFA clusters. *H. parainfluenzae* and *H. influenzae* isolates from urine were not associated with concurrent respiratory infection.

Conclusion: REP-PCR and CFA pattern analysis can distinguish a cryptic group of urinary tract isolates of *Haemophilus parainfluenzae* that can be clinically significant.

P1106 Polish National External Quality Assesment Scheme for Microbiology (POLMICRO)

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Objectives: Microbiology Laboratories throughout the world participate in external quality assessment schemes, often as a prerequisite for accreditation. The POLMICRO established in December 1993 provides a quality assessment scheme service in bacteriology and antibiotic susceptibility testing (AST).

Methods: Participation was voluntary although it was strongly recommended, especially for laboratories chaired by district consultants. 5–6 samples for bacterial identification and antibiotic susceptibility testing were dispatched in each distribution. Participants were given one month to examine and return the results. All participating laboratories were informed about their overall performance and where necessary comments were made in an effort to improve the standards of testing.

Results: The number of participants in the POLMICRO varied from 81 to 100 laboratories. Over the three assessment periods the percentage of participants returning reports that were considered satisfactory was increased from 27% to 76%. Results of identification were generally acceptable except for *Streptococcus pneumoniae*. Most discrepancies in the scheme were in the area of AST. This was presumably because there are more technical and interpretative procedures. About 50% of participants in 1993 and 1995, and 6% in 1996 failed to detect methicllin resistance of *S. aureus*. The error rates seen in the last two years with penicillin-resistant *S. pneumoniae* and with enterococci highly resistant to aminoglycosides were found to be 13% and 26%, respectively.

Conclusion: During the three year period an important increase in quality of microbiology laboratory performance was observed.

Chlamydia and ureaplasma

P1107 A Novel Assay that Detects and Differentiates *Chlamydia trachomatis* and *Neisseria gonorrhoeae* Using Transcription Mediated Amplification

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Objective: Develop an assay that detects and differentiates both *Neisseria gonorrhoeae* and *Chlamydia trachomatis* rRNA from male and female urine and swab specimens.

Methods: Target rRNA is captured out of matrix onto magnetic particles as a sample processing method (target capture) that effectively removes inhibitors of nucleic acid amplification. The target rRNA is amplified by Transcription Mediated Amplification (TMA). The amplification products are hybridized with two different acridinium ester-labeled oligonucleotides that are each specific for the two target organisms. Kinetic differences in the light-off reactions of the two acridinium ester labels, following a hybridization protection assay, allow for the deconvolution of the signal, and the detection of the two analytes in one assay.

Results: Male and female urine samples (n = 101) were tested in a blind study. The assay had a sensitivity of 96.4% (27/28), and a specificity of 100% for *Chlamydia trachomatis* against the Gen-Probe AMP CT assay. Fifty five endocervical swabs were assayed with Gen-Probe's PACE 2 assay as the reference. The dual analyte assay was 100% sensitive (8/8), and 97.9% specific (46/47) for *Chlamydia trachomatis*. In 10% blood in urine, at the single organism target rRNA level, the TMA reaction was not inhibited following target capture. In the presence of one million *Neisseria meningitidis* cells, the target level equivalent of a single *Neisseria gonorrhoeae* cell could be detected. A urine transport buffer compatible with this assay has been defined.

Conclusion: This assay is being designed in concert with an instrument that totally automates sample processing, amplification, and detection.

P1108 Single-Test Detection of Amplified rRNA from *Chlamydia trachomatis* and *Neisseria gonorrhoeae* on the VIDAS Instrument

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A single, rapid and sensitive automated test for the simultaneous detection of *Chlamydia trachomatis* (Ct) and *Neisseria gonorrhoeae* (Ng) is being developed on the VIDAS. This test uses spatially separated probes bound to the VIDAS dispensing tip (called the SPR) for specifically capturing Ct and Ng rRNA sequences amplified by the transcription-mediated amplification (TMA) technology. If both organisms are present in a sample, the rRNA from each is co-amplified in a single reaction and individually detected in one VIDAS test strip. Signal is produced from alkaline phosphatase labeled probes which are specific for each amplicon and hybridize to their respective targets during the single capture hybridization step. Total assay time, including amplification and detection (but excluding sample processing steps), is about three hours. Discrete results are generated for the presence or absence of each target sequence. In model assays using purified rRNA molecules, the sensitivity of this test was about 100 Ct rRNAs (equivalent to less than one elementary body) and 4000 Ng rRNAs (equivalent to roughly two cells). These sensitiv-